domain. Myosin-7a unfolds at either high ionic strength or in the absence of ATP, revealing a clearly recognizable motor domain, the lever arm and some features of the tail region. C-terminal truncations were made to determine which portions of the multi-domained tail are necessary for the regulation. Removal of the last 99 amino acids which are highly conserved in all myosin-7a molecules and form a subdomain (termed MyTH7) of the FERM domain, or mutation of two conserved amino acids in this region, is sufficient to prevent folding of the molecule in the presence of ATP and activates the enzymatic activity. A construct consisting of the second FERM domain binds actin in an ATP-insensitive manner with a Kd of 30μM which is similar to the KATPase value for the full length molecule. We propose that at low actin concentration myosin-7a is folded and inactive, but at high actin concentrations such exists in actin bundles, it binds first via its tail binding site which then frees the motor domain to functionally interact with actin.

18-Symp
The Ups and Downs of Smooth Muscle Myosin Regulation
Kenneth A. Taylor.
Florida State University, Tallahassee, FL, USA.
Since discovery that regulatory light chain (RLC) phosphorylation was the primary method of regulation of smooth and non-muscle myosin II, the structure of the phosphorylated and dephosphorylated forms has been a goal only partially realized. Regulation in both smooth muscle and the related scallop striated muscle myosin requires a 2-headed myosin species; single headed species are unregulated. Thus head to head interactions are key to achieving the inhibited state. A folded conformation of full length myosin, generally referred to as the 10S conformation was discovered early by conventional electron microscopy and in 1999, a structural explanation for the head-head interactions was obtained from 2-D arrays of dephosphorylated smooth muscle heavy meromyosin formed on lipid monolayers and imaged in 3-D by cryoEM. The structure was later confirmed by 2-D arrays of the 10S conformation of whole myosin. While this structure explained inhibition of the solubilized form of regulated myosins, it had not been observed in filaments. Surprisingly, the first observation of smooth muscle myosin-like head-head interactions in a thick filament was obtained from tarantula striated muscle, not from smooth muscle myosin. Even more surprising was the observation of a similar conformation in relaxed cardiac muscle thick filaments. Thus, these head-head interactions observed first in smooth muscle HMM, appear to be ubiquitous in relaxed muscle although still not confirmed for thick filaments in relaxed smooth muscle. Still to be determined is an explanation of the factors that can lead to solubilization and the location of the N-terminus of the RLC, whose location and structure has yet to be revealed, in the phosphorylated and dephosphorylated state. Theoretical modeling has provided possible explanations for several factors that affect regulation but has not yet yielded a coherent theory. Supported by NIAMSD.

19-Symp
Switching Gears with Myosin Binding Protein-C
Samantha P. Harris[1], Justin F. Shaffer[1,2], Kristina L. Bezold[1], Robert W. Kensler[3].
[1]University of California, Davis, Davis, CA, USA, [2]University of Washington, Seattle, WA, USA, [3]University of Puerto Rico, San Juan, PR, USA.
Myosin binding protein-C (MyBP-C) is a thick-filament protein in vertebrate sarcomeres that limits cross-bridge cycling kinetics and reduces myocyte power output. However, the mechanisms by which MyBP-C influences cross-bridge kinetics are not well understood. The goal of the present study was to investigate the ability of the first 4 N-terminal domains (C0-C1-motif-C2) of cardiac (c) MyBP-C to affect actomyosin interactions and interact with actin. Here we show that recombinant proteins containing the C1 and motif domains increased Ca^{2+} sensitivity of tension and increased rates of tension redevelopment (k_{tr}) at submaximal [Ca^{2+}] in permeabilized rat trabeculae. Proteins containing these domains also biphasically activated then inhibited Ca^{2+}-activated ATPase rates of heavy meromyosin and myosin S1 in solution. Cosedimentation binding assays demonstrated saturable binding of the 4 N-terminal domains to F-actin at a 1:1 molar ratio (Kd ~ 10μM). However, more than one interaction site was indicated by turbidity and electron microscope analyses that showed actin bundling in the presence of recombinant proteins. Phosphorylation of the motif or increasing pH reduced binding to a 1:2 molar ratio and abolished actin bundling. Phosphorylation reduced but did not eliminate effects of recombinant proteins to increase Ca^{2+} sensitivity of tension and k_{tr} at submaximal [Ca^{2+}] in permeabilized trabeculae. Together these results suggest that the N-terminus of cMyBP-C interacts with F-actin through multiple distinct sites, at least one site is modulated by electrostatic charge interactions, and that functional effects of the N-terminus of MyBP-C are mediated in part by phosphorylation independent mechanisms. Supported by NIH HL080367.

Platform A: Protein Conformation

20-Plat
Active Unfolding of Collagen is not Required for Collagenolysis to Occur in Solution
Ramon Salsas-Escat, Collin M. Stultz.
Massachusetts Institute of Technology, Cambridge, MA, USA.
A number of disorders such as tumor metastasis, arthritis, and atherosclerotic heart disease are related to excessive collagen degradation. Therefore methods that further our understanding of collagenolysis are of particular interest. However, as the collagenase active site is too small to accommodate the triple-helical structure of collagen and the scissile bond within collagen is not accessible to the collagenase catalytic site, the precise molecular mechanism of collagenolysis is unclear. Prior experiments have been interpreted to mean that collagenases actively unfold collagen, in a process that requires intact full length collagenase - which is typically a multi-domain protein, containing both a catalytic and hemopexin-like domain - leading to exposure of the scissile bond. Here we demonstrate that collagen types I and III can be degraded by the catalytic domain alone of either MMP1 or MMP8 at temperatures far below the melting temperature of collagen. These data argue that active unwinding of collagen is not required for collagenolysis to occur in solution. Molecular simulations further suggest that normal thermal fluctuations in the structure of the triple-helical structure of collagen lead to the protein sampling states where the scissile bond is relatively exposed and hence accessible to collagenase active site. Taken together, these data suggest that collagen degradation is the result of the interaction of preformed locally unfolded states of collagen and collagenases, rather than a mechanism that involves active collagenase-mediated unfolding.

21-Plat
Lipid Bilayer Coated Gold Nanoparticles Provide Insight Into Proteins' Conformational Changes
Stephan M. Woods[1], Katrice E. King[1], Avishek Kumar[2], Roberta E. Redfern[1], Alonzo Ross[3], Arne Gericke[1].
[1]Kent State University, Kent, OH, USA, [2]Carnegie Mellon, Pittsburgh, PA, USA, [3]University of Massachusetts Medical School, Worcester, MA, USA.
While nanoparticles have been studied and used for many years and their unique chemical and physical properties have been extensively characterized, nanoparticles are now increasingly used to investigate biological systems. Nanoparticles can be formed on a submicron scale and do not interfere with normal biological processes, thus can be used in an *in vivo* system, allowing researchers to gain insight into its inner workings. The use of nanoparticles has also proven to be quite flexible, and has provided an indispensable tool in the advancement of drug delivery, tissue engineering, and detection of biomolecules. We have previously studied conformational changes occuring within proteins upon binding to phospholipid model membranes, particularly the tumor suppressor protein PTEN. To gain a more in depth characterization of these protein conformation changes, we have developed a technique in which we have fabricated 50nm gold nanoparticles coated with an asymmetric lipid bilayer, in a manner that allows us to control the identity of the lipids making up the outer leaflet. Using Raman spectroscopy, we will take advantage of the surface enhanced Raman spectroscopy (SERS) effect, which will increase PTEN band intensities binding of the protein to the lipid covered nanoparticle. Systematic mutation of tryptophan residues within the protein will allow us to probe the binding induced conformational changes in the vicinity of the tryptophan. While SERS has been used previously to study protein conformational changes, proteins were in these cases directly immobilized onto the nanoparticles. In contrast, our novel approach immobilizes the lipids on the nanoparticles and the protein can freely interact with the target lipid. While we plan to use these methods to examine PTEN, further development of this technique will allow for better study of conformational changes in a myriad of interfacial enzymes.

22-Plat
Extended conformations in alanine peptides
Kang Chen[1], Zhigang Liu[1], Chunhui Zhou[1], W. Clay Bracken[2], **Neville R. Kallenbach**[1].
[1]New York University, New York, PA, USA, [2]Weill Cornell College of Medicine, New York, NY, USA.
Spectroscopic evidence for the presence of local order in unfolded proteins, including polyproline II (PII) structure, now appears incontrovertible. The data supporting this order relies on analysis of short chain peptides. The dimensions of unfolded chains nevertheless conform to random coils. We have re-examined the dimensional properties of short chains using paramagnetic proton spin relaxation measurements to evaluate intermediate range distances (r)

within alanine peptides linked to short proline arms.(1) Two peptides were employed in our studies,, OO-T*-PPPA*PPPA*-OO and OO-T*-PPPAAAA*-OO, where O is Ornithine, T* is Toac (2,2,6,6-Tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid), A* is 15N labelled alanine. Mesostate based Monte-Carlo sampling calculations (2) were carried out to interpret the relaxation data. The conclusion of the study is that over 90% occupation of extended conformations, i.e. PII and β mesostates, is required to reproduce the experimentally observed distance averaging. Compact structures including αR, αL and turns are clearly present but are not dominant in the conformational ensemble. Analysis of the conformation of other side chains will be presented.

(1) Chen, K et al. Ang. Chem. Int. Ed. 2007 46, 9036.
(2) Gong, H, Fleming, PH, Rose, GD. Proc. Natl. Acad. Sci. USA 2005, 102, 16227.

23-Plat

A Solution NMR and Crystallographic Study of the Role of the Quaternary Shift in the Allosteric Regulation of Phosphofructokinase from B. stearothermophilus

Rockann Mosser, Manchi Reddy, James C. Sacchettini, Tatyana Igumenova, Gregory D. Reinhart.
Texas A&M Univ, College Station, TX, USA.

The 136 kDa Bacillus stearothermophilus phosphofructokinase (BsPFK) is a homotetramer that is allosterically inhibited by phospho(enol)pyruvate (PEP), which binds along one dimer-dimer interface. Fru-6-P binds along the other dimer-dimer interface. The substrate bound and the inhibitor bound structures of wild-type BsPFK exhibit a 7° rotation about the substrate binding site interface, termed the quaternary shift. The binding of substrate and inhibitor to BsPFK have been studied using methyl TROSY NMR. By selectively labeling all 30 Ile residues in the BsPFK monomer with 13CH3 in an otherwise fully deuterated enzyme, only the Ile are detected on a 2D-1H-13C correlation spectrum. Several distinct Ile cross-peaks change position when PEP is added to wild-type apo BsPFK. To distinguish between changes associated with the quaternary shift and those associated with intra-subunit tertiary changes, the variant D12A BsPFK is currently being studied using kinetics, x-ray crystallography, and methyl TROSY NMR. When compared to wild-type, D12A BsPFK shows a 100-fold increase in the binding affinity for PEP, a 50-fold decrease in the binding affinity for F6P, and an allosteric coupling comparable to wild-type. Crystal structures of apo and PEP bound forms of D12A BsPFK both indicate a shifted structure similar to the inhibitor bound structure of wild-type. Since PEP still inhibits D12A BsPFK substantially despite the fact that it has already adopted the inhibited quaternary structure, the inhibition likely involves further tertiary changes to the enzyme structure. NMR of deuterated, 13CH3 labeled D12A is being performed in an effort to identify residues involved in these tertiary changes. Supported by grant GM33216 from NIH and grant A1543 from the Welch Foundation.

24-Plat

From Data or Dogma? The Myth of the Ideal Helix

Daniel J. Kuster, Sergio Urahata, Jay W. Ponder, Garland R. Marshall.
Washington University in St. Louis, Saint Louis, MO, USA.

In the course of building detailed surface representations for peptidomimetics, we were motivated to analyze the detailed surfaces of helices in proteins. Unexpectedly, we observed few ideal helical forms in high-resolution protein structures. Instead of a bimodal distribution matching the well-known alpha and 3(10) helical forms, we observed a smooth, single-peaked population, characterized by intermediate helices with shared hydrogen bonds. Bifurcated, or three-center hydrogen bonds, have been well-documented in small molecules and peptides, but they're rarely highlighted in the context of folded proteins. The data suggests shared hydrogen bonds are a major component of helices in proteins. Contrary to the Pauling-Corey-Branson models, we did not restrict our analysis to single hydrogen bonds–shared three-center hydrogen bonds were included. High resolution (<2.0 Å), electron density data is sharp enough that a helical structure should be unambiguous and accurately modeled. These helices are centered on an intermediate helical form. At poorer resolutions (2.0-5.0 Å), electron density is ambiguous; refinement fitting methods are employed to model ideal structures into the data. Here there is an enriched population of ideal structures. A structurally representative subset of proteins reveals the same trends as the whole PDB. The data support the observation that ideal helical parameters do not accurately describe the distribution of real helices in proteins. Hydrogen bonds are a polar (and polarizable) moiety and an accurate model must account for this. We present data comparing molecular dynamics simulations using popular monopole force fields (OPLS-AA and CHARMM) with a next generation force field (AMOEBA) implementing polarizability and multipole electrostatics. AMOEBA simulations are shown to quantitatively reproduce the experimentally observed trends in helical populations. These results emphasize the importance of using appropriate force field potential models when simulating hydrogen bonded structures in proteins.

25-Plat

Conformation Coupling Between The I-like Domain Alpha7 Helix And The Hybrid Domain Of Beta3 Integrins

Jizhong Lou, Wei Chen, Mei-Yin Chou, Cheng Zhu.
Georgia Institute of Technology, Atlanta, GA, USA.

Integrins are αβ heterodimers that mediate cell adhesion and transduce signals bidirectionally across the cell membrane. Integrins often exist in low affinity (or inactive) states for ligand binding on the cell curface, but change conformations to high affinity (or activated) states when induced by stimuli from inside or outside the cell. Crystallographic and electron microscopic studies have obtained evidence that the low affinity states correspond to bent conformations where the integrin headpiece interacts with the legs, whereas the high affinity states correspond to more extended conformations where integrins stand up, suggesting a switchblade-like unbending model for integrin activation. In this model, the key conformational changes on integrin headpiece include the swing out of the hybrid domain and the downward movement of I-like domain α_7 helix. These conformational changes are suggested by crystal structures of β_3 integrin headpieces. The unliganded $\alpha_v\beta_3$ headpiece displays a closed hybrid domain with the I-like α_7 helix in an upper position and the pseudo-liganded $\alpha_{IIb}\beta_3$ headpiece shows an open hybrid domain with the I-like α_7 helix in a lower position. Using molecular dynamics simulations we studied the stability of the two conformations of the I-like domain α_7 helix and the hybrid domain, the transition between the two conformations in each structure, and the coupling between the conformational changes in the two structures. We observed that the β-propeller domain of the α subunit helped stabilize the hybrid domain at the close conformation. The down/upward movements of the I-like α_7 helix correlated with the opening/closing of the hybrid domain. Our simulations identify key residues to the α_7 helix movement and suggest the existence of intermediate conformations of the hybrid domain between the open and the close conformations.

26-Plat

Spin-label EPR of alpha-Synuclein on Vesicles Reveals Antiparallel Arrangement and Differences in the Membrane Binding Affinity of the two Helices

Martina Huber[1], Malte Drescher[1], Frans Godschalk[1], Sergey Milikisyants[1], Gertjan Veldhuis[2], Bart van Rooijen[2], Vinod Subramaniam[2].
[1]Leiden University, Leiden, Netherlands, [2]TwenteUniversity, Enschede, Netherlands.

The Parkinson's disease-related protein α-Synuclein (αS) is a 140 residue protein that is natively unfolded in solution. Its membrane-binding properties are implied in its physiological or pathologic activity. αS was investigated by spin-label EPR. By Electron-Electron Double Resonance (DEER) the distance between the spinlabels in four double mutants was determined in the vesicle-bound and free form of αS, revealing antiparallel arrangement of the helices. Thus, even in the vesicle-bound form αS has the horseshoe conformation, revealing that this conformation is intrinsic to the protein, rather than induced by the small size of micelles investigated previously. Mobility analysis of five single spin-labeled mutants showed that the membrane affinity of helix 2, comprising residues 45 - 90, decreases with decreasing negative charge of the membrane surface, suggesting differential binding of αS to membranes. The findings reveal molecular details of the membrane-bound conformation of αS not previously obtained.

27-Plat

A Coarsened Network Model Reveals Allosteric Machinery

Ali Rana Atilgan, Canan Atilgan.
Sabanci University, Istanbul, Turkey.

The past decade has witnessed the development and success of coarse-grained network models of proteins for predicting many equilibrium properties related to collective modes of motion(1,2). We first use a systematic approach on a large set of globular proteins to find a basis for why the network models work well to define certain properties of the system. The analysis is based on the radial distribution function and the spectral dimensions(3) of proteins as well as newly defined quantities, the angular distribution function, and the contact order per mean path length of individual residues. We prove that the network construction is free of the cut-off distance problem if one is interested in the collective motions of the residues.

We next undertake a linear response analysis. If the collection of forces applied on a specific residue is independent and large in number, they will appear in a spherically symmetric set of directions. With the aid of responses deviating from such a spherically symmetric distribution, we rigorously determine the residues involved in the remote control of the ligand. These are usually charged surface loop residues, providing binding locations for ions which are known to influence ligand release kinetics(4). We prove that by perturbing any one of these residues, the tip of the cap that opens the exit of the ligand is made to operate coherently, irrespective of the direction of the perturbation. We thereby